as that provided in the previous actions. In addition, in response to Applicants' clarification submitted in the previous response, the Examiner states that the various functions or biological activities disclosed in the specification (e.g., binding to hyaluronate) are not specific to the sequences recited in the claims. Labeling the disclosed biological activities as "prophetic," the Examiner further states that such disclosure does not arise to patentable utility because there is no available evidence which indicates that the disclosed interactions/functions occurred amongst the claimed sequences or IPM molecules in general. These rejections are respectfully traversed for the following reasons.

I. The present disclosure and specific utilities of the claimed sequences

1. All evidence indicate practical utilities of the IPM sequences

The prior art knowledge and the disclosure of the subject application all suggest that the IPM sequences are involved in retinal adhesion and ocular disorders. It is known that the IPM sequences (e.g., IPM 150) is selectively expressed in the retinal tissue (see, e.g., Felbor et al., Cytogene. Cell Genet. 81:12-17, 1998, at page 16, left column; copy attached). The subject specification disclosed that IPMC proteins (e.g., IPM150) contain hyaluronan-binding motifs and that IPM150 could interact with hyaluronan, a component of the interphotoreceptor matrix, to effect retinal adhesion (see, e.g., page 20, line 28-30; and page 21, lines 3-9). It was also taught in the subject specification that the IPM proteins also contain EGF-like domains. Although EGF-like domains may be present in proteins with diverse function, as noted by the Examiner, it does not negate the fact that they are present in many extracellular matrix proteins and are known to promote the survival of neighboring cells.

The IPM sequences are also genetically linked to a number of macular dystrophies. For example, Felbor et al. indicated that IMPG1 (i.e., IPM150) is a candidate for retiniopathies (see, e.g., the title and the abstract). The authors specifically noted that "the selective expression in retinal tissue and the chromosomal mapping of IMPG1 to 6q13-q15 have identified this gene as an attractive candidate for several human macular dystrophies . . ." (see, page 16, left column). Similarly, the subject specification disclosed (see, e.g., page 8,

lines 16-21) that the IPM 150 sequence is mapped to the 6q14.2-q15 region which also contains loci for progressive bifocal chorioretinal atrophy, autosomal dominant Stargardt's-like macular dystrophy, North Carolina macular dystrophy and Salla disease.

Based on the present disclosure and the prior art knowledge, there is no doubt that the present invention can have practical and useful applications. For example, as disclosed in the specification, they could find applications in diagnosing (e.g., by detecting a mutation in the IPM molecules or an abnormal expression of the IPM molecules) and treating (e.g., in gene therapy) ocular disorders that are associated with abnormal retinal adhesion, such as retinal detachment and macular degeneration.

2. The disclosed utilities are specific, not general

According to the MPEP, a "specific" utility is specific to the subject matter claimed. It is in contrast to a general utility which would be applicable to the broad class of invention (MPEP § 2107.01-I, at page 2100-32). The MPEP also sets forth exemplified circumstances under which a specific utility is not present. These examples include (i) disclosing a compound which may be useful in treating unspecified disorders; (ii) claiming a polynucleotide whose use is disclosed simply as a "gene probe" or "chromosome marker"; and (iii) a general statement of diagnostic utility, such as diagnosing an unspecified disease (MPEP, § 2107.01 at page 2100-32).

Clearly, the present invention does not fall into any of the above categories which only disclose general utilities. As discussed above, the specific utilities of the presently claimed IPM sequences are substantiated by, e.g., their selective expression in retinal tissue and their genetic linkage to certain specific ocular diseases. The fact that IPM150 is believed by the skilled artisans (see, e.g., Felbor et al., supra) to be the candidate locus for retiniopathies also undoubtedly underscores the specificity of the practical utilities of the presently claimed sequences. Thus, rather than unspecified disorders or merely as gene probe (i.e., general utility), the practical utilities disclosed in the subject specification are specific to the IPM sequences.

3. Specific utility does not mean exact or unique biochemical/physiological function

In maintaining the instant rejection, the Examiner apparently takes the position that only the exact and experimentally proven physiological functions of the IPM polypeptides would satisfy the utility requirement. However, such is not the legal test for a specific utility. To the contrary, as illustrated in the MPEP (e.g., § 2107.03-I, at page 2100-43), the utility requirement only mandates a reasonable correlation between a disclosed biological activity (e.g., selective expression in retinal tissue and hyaluronan-binding) and a disease state (e.g., retiniopathies). It does not require conclusive proof that the disclosed biological activity is causatively linked to the disease state.

Applying the above standard to the instant case, it is clear that the subject specification has undoubtedly disclosed specific utilities that satisfy the requirement of the utility guidelines. The present disclosure in combination with the prior art taught that the IPM 150 and IPM 200 proteoglycan sequences are important in maintaining retinal adhesion (e.g., through its hyaluronan-binding motifs) and can be involved in a number of ocular disorders. Their selective expression in retinal tissue and biochemical properties (e.g., hyaluronan-binding motifs), as well as their chromosomal mapping to a loci that is genetically linked to a number of macular diseases or disorders, have provided the reasonable correlation between the disclosed biological activity and the disease state. It is respectfully submitted that there is no requirement in the Utility Guidelines that only proven physiological roles of a protein encoded by a new gene would satisfy the specific utility test.

II. Additional real-world utilities that would have been readily apparent

Accordingly to the Utility Guidelines, the utility requirement can be satisfied if a patentable utility is readily apparent from the disclosure. See, *Federal Register*, Vol. 66, No. 4, at page 1095, left column, Comment 11 (2001). Applicants note that, in addition to the above-discussed utilities specifically set forth in the specification, the skilled artisans would

also appreciate other utilities that would have been readily apparent from the present disclosure. For example, the novel polynucleotide sequences identified by the present inventors can be readily applied in polynucleotide array technology. Polynucleotide arrays are commercially available and have been widely used by the skilled persons in the art. Such arrays typically contain oligonucleotide or cDNA probes to allow detection of large numbers of mRNAs within a mixture. They are often used to study differential gene expression and to analyze candidate drugs for roles in modulation of a disease state.

Thus, it would have been apparent that the IPM sequences are useful for inclusion on a polynucleotide array (e.g., an Affymetrix GeneChip™ array or the like) together with probes containing a variety of other genes. With increased diversity of probe sequences, the modified arrays provide improved tools for the various applications of polynucleotide arrays. Such improved arrays are particularly useful in analyzing ocular tissues or cells. The IPMC polynucleotide sequences can also be combined with nucleic acids from other genes having roles in ocular diseases or disorders (e.g., as described in the subject specification) in an array that are specifically designed for analyzing ocular disease related gene expression. Such arrays are useful for analyzing and diagnosing cells in ocular diseases such as retinal detachment. Such arrays are also useful for analyzing candidate drugs for roles in modulation of an ocular disease state. No one would doubt that such applications of the present invention constitute credible, substantial, and real-world utilities.

For all the above reasons, Applicants submit that the presently claimed invention has a patentable utility that satisfies the requirement of 35 U.S.C. 101. Therefore, the rejections under 35 U.S.C. §§ 101 and 112 should be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400 x 5209.

Respectfully submitted,

Hugh Wang
Reg. No. 47,163

Attachment: Felbor et al., Cytogene. Cell Genet. 81:12-17, 1998

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Cytogenetics and Cell Genetics

Genomic organization and chromosomal localization of the interphotoreceptor matrix proteoglycan-1 (IMPG1) gene: a candidate for 6q-linked retinopathies

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Abstract. The interphotoreceptor matrix is a unique extracellular matrix occupying the space between the photoreceptors and the retinal pigment epithelium. Due to its putative function in the maintenance and integrity of the photoreceptor cells, it is conceivable that it is involved in retinal degeneration processes. More recently, a novel gene encoding a 150-kDa interphotoreceptor matrix proteoglycan, designated IMPG1, was cloned and shown to be expressed in both rod and cone photoreceptor cells. To assess this gene in human retinal dystrophies, we have now determined the genomic organization and chromosome location of IMPG1. It is composed of 17 exons ranging from 21 to 533 bp, including an alternatively spliced exon 2. Using somatic cell hybrid mapping and FISH analysis, we have assigned the IMPG1 locus to 6q13→q15. As this interval overlaps with the chromosomal loci of several human retinopathies, including autosomal dominant Stargardt-like macular dystrophy (STGD3), progressive bifocal chorioretinal atrophy (PBCRA), and North Carolina macular dystrophy (MCDR1), IMPG1 represents an attractive candidate for these 6q-linked disorders.

The interphotoreceptor matrix (IPM) is a unique extracellular matrix located in the subretinal space between the neural retina and the retinal pigment epithelium (RPE). It has been implicated in photoreceptor cell-supportive functions by mediating interactions between the photoreceptors, the RPE, and Müller cells (Hewitt and Adler, 1989; Hageman and Johnson, 1991). In addition, IPM proteoglycans are thought to participate in the maintenance of normal retina-RPE adhesion and the integrity of cone photoreceptor cell outer segments (Yao et al., 1990; Lazarus and Hageman, 1992).

Several studies of retinal degeneration in animal models have addressed the possibility of disturbed cell-IPM interactions in photoreceptor degeneration (LaVail et al., 1993; Lazarus et al., 1993; Mieziewska et al., 1993a, b). In the progressive rod-cone degeneration miniature poodle (Mieziewska et al., 1993b), the murine autosomal recessive nervous mutation (LaVail et al., 1993), and the rod-cone dysplasia 1 Irish setter (Mieziewska et al., 1993a), progressive photoreceptor degeneration occurs slowly, with rods being affected earlier and more severely than cones. As compartmentalization of the IPM is most obvious in the so-called cone matrix sheaths (Johnson et al., 1986), a correlation between the degenerative processes in these animals and the integrity of specific IPM domains has been suggested, although the nature of this relationship remains unclear (Mieziewska et al., 1993a, b; LaVail et al., 1993). Furthermore, prior to photoreceptor loss in mice affected with mucopolysaccharidosis type VII, an altered distribution of IPM chondroitin 6-sulfate containing proteoglycan has been observed (Lazarus et al., 1993). It is noteworthy that retinal degeneration is also known to occur in humans affected with mucopolysaccharidoses, a heterogeneous group of lysosomal storage diseases caused by a deficiency of one of the key enzymes required for glycosaminoglycan degradation (Gills et al., 1965; Goldberg and Duke, 1967).

Chondroitin 6-sulfate containing glycoconjugates constitute a major component of the cone matrix sheaths (Hageman and

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Johnson, 1987). More recently, the full length cDNA of IMPG1 encoding a 150-kDa chondroitin 6-sulfate proteoglycan was cloned (Kuehn and Hageman, 1995) and shown to be expressed in rod and cone photoreceptor cells (Kuehn et al., 1997). As part of our search for genes involved in human retinal disorders, we have determined the expression profile, genomic organization, and chromosomal localization of IMPG1 as a prerequisite for its mutational analysis in human retinopathies.

Materials and methods

Northern blot analysis

Total RNA was isolated from human lung, cerebellum, and retina and the human RPE cell line ARPE19 (Dunn et al., 1996), using the RNA-Clean-LS system (Angewandte Gentechnologie Systeme). The Northern blot contained 12 µg of total RNA in each lane. Hybridization with a radiolabeled 1,166-bp cDNA fragment was performed at 65 °C in 0.5 M $NaPO_4$ (pH 7.2), 7% SDS, and 1 mM EDTA (pH 8.0) (Church and Gilbert, 1984). The 1,166-bp cDNA fragment was PCR amplified from first stranded retinal cDNA using a Superscript Kit (GIBCO BRL) and oligonucleotide primers IPM5'F/IPM1399 designed according to the full-length IMPG1 cDNA sequence (Kuehn and Hageman, 1995).

Genomic and cDNA library

A human genomic PAC library (RPCI1) gridded in 321 individual 384-well microtiter plates was generously provided by Dr. Pieter de Jong (Roswell Park Cancer Institute, Buffalo, NY). For library screenings, the 1,166-bp cDNA PCR product was double-digested with *Hin*cII and *Eco*RI, resulting in a 515-bp fragment corresponding to the 5' region of the gene. In addition, a 580-bp PCR probe was amplified from the 3' end of IMPG1 with the primers C6SP-a/C6SP-m. PAC clone DNAs were obtained by the conventional alkaline lysis procedure. To establish overlaps between the isolated PAC clones DOP end-fragment-vector PCR was performed as described in Wu et al. (1996). A retinal cDNA library was kindly provided by Dr. J. Nathans, Johns Hopkins University, Baltimore, MD.

PAC subcloning

PAC clones dJ47C10 and dJ38F21 were digested with *Hin*dIII, *Eco*RI, and *Sau*3AI and subcloned into pBluescript II KS (+) phagemid vector (Stratagene). Exon-containing subclones were identified by colony filter hybridization with a 2,975-bp PCR fragment (1F/C6SP-m) and partially sequenced using the dideoxy chain termination method (Sequenas Version 2.0 DNA sequencing kit; US Biochemical) and internal oligonucleotides as given below. Exon/intron boundaries were identified by alignment of the genomic sequences with the published IMPG1 cDNA sequence (Kuehn and Hageman, 1995) using MacVector sequence analysis software (release 4.0).

Data analysis and oligonucleotide primers

To search for expressed sequence tags (ESTs) in the available databases, the BLASTN program of the GCG Package was utilized (Genetics Computer Group, 1996). The following oligonucleotide primers were used in this study: IPM5'F: 5'-TAG ACA ATC CCC AAG AAA TG-3' (cDNA nucleotide [nt] position: +107 to +127); 1F: 5'-AGA TTT GAG GTT GTT CTG TG-3' (nt −55 to −36); IPM605: 5'-AGA GAA GTT TCC CTG ACA G-3' (nt +479 to +497); IPM789: 5'-TGT AGG CAT CTT GGT GTC G-3' (nt +645 to +663); IPM947: 5'-TTA AGA AAC TTC CAG GAT TC-3' (nt +821 to +840); IPM1328: 5'-CAG CAA AAG ATG TGG GCA G-3' (nt +1,183 to +1,201); IPM1399: 5'-CTC CGT CCA CTG TCT CAA GC-3' (nt +1,254 to +1,273); C6SP-a: 5'-ATT ACT GAC CGT AGA ATA TG-3' (nt +2,340 to −2,359); C6SP-m: 5'-GAG GTT TGT GTT TAT CAG AC-3' (nt −2,901 to + 2,920); M13f5: 5'-CGC CAG GGT TTT CCC AGT CAC GAC-3'; and M13r6: 5'-AGC GGA TAA CAA TTT CAC ACA GGA-3'.

Hybrid panel PCR

PCR was performed with a commercially available panel of 25 human × hamster hybrid cell line DNAs (BIOS Corporation) and oligonucleotide primers C6SP-a/C6SP-m. Giemsa banding was used to verify the presence of the correct human chromosomes.

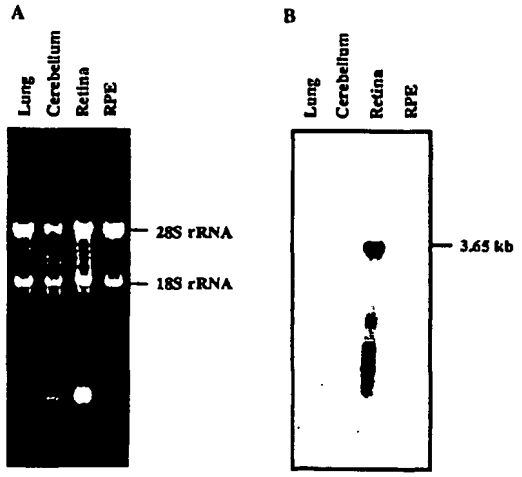

Fig. 1. Northern blot analysis of IMPG1. **(A)** Ethidium bromide-stained agarose gel showing the approximate amount of total human RNA loaded onto each lane. **(B)** Northern blot hybridization of the 1,166-bp IMPG1 cDNA fragment. An abundant 3.65-kb transcript is observed in retina. There are no signals in lung, cerebellum, or retinal pigment epithelium (RPE) total RNA.

Fluorescence in situ hybridization (FISH)

Metaphase chromosomes from peripheral blood lymphocytes were prepared using a standard 1:3 *(v/v)* acetic acid:methanol fixation protocol. Procedures for removal of interfering remnants of cellular RNA and cytoplasm, as well as standard methods for biotinylation of PAC dJ38F21 and FISH, were described elsewhere (Köhler and Vogt, 1994). To determine the cytogenetic band position of the IMPG1 gene, faint Q-bands observed after DAPI staining (Schweizer, 1976) of more than 25 metaphases were related to the position of hybridization signals on propidium-iodide counterstained chromosomes. In addition, the distance from centromere to signals was measured relative to the overall chromosome arm length. The ideogram established by Francke (1994) was used as a reference.

Results

Database analysis and expression profile of IMPG1

Alignment of IMPG1 cDNA sequences to the GenBank and dbEST databases revealed significant identity to ESTs yp48c06 (H38839, H38594), yp48e04.r1 (H38604), and 16h10 (W26960). These cDNA clones have been isolated exclusively from human retinal cDNA libraries (Soares et al. 1994). To analyze the expression pattern of IMPG1 in various adult human tissues, Northern blot analyses were performed with total human RNA isolated from retinal pigment epithelium, retina, lung, and cerebellum. Filter hybridization with probe IPM5'F/IPM1399 corresponding to nt 107 to 1,273 of the full-length cDNA revealed a 3.65-kb transcript in total retinal RNA (Fig. 1). No hybridization signals were detected in RNA from retinal pigment epithelium, lung, or cerebellum even after overexposure of the autoradiogram.

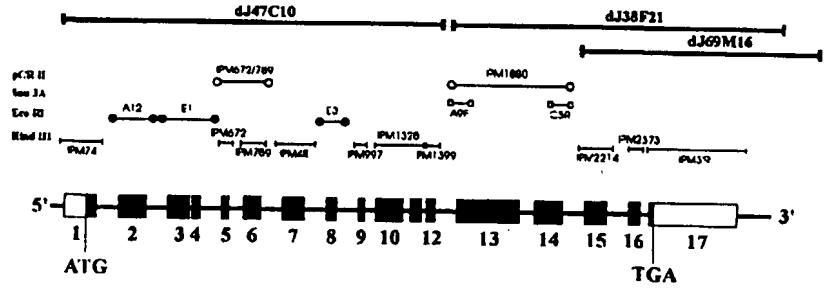

Fig. 2. Genomic organization of the IMPG1 gene. The top portion of the figure shows three PAC clones (dJ47C10, dJ38F21, and dJ69M16) together containing the entire coding sequence of the gene. The horizontal bars below depict PAC-derived plasmid subclones used in the sequencing analysis. Boxes represent exons 1 to 17; 5'- and 3'-flanking regions are shown as open boxes. Intron sizes are drawn to scale with the same size line for those that are over 4 kb in length.

Table 1. Exon/intron boundaries of the human IMPG1 gene

Splice acceptor	Score[a]	Exon No.	Coding sequence (bp)	Splice donor	Score	Intron (kb)	Intron phase[b]
–	–	1	67	AAGGTAAGT	0.2	>4	I
AATCTTTCTTTTACAGA	3.9	2	234	GAGGTAAGG	1.7	>4	I
TGACTCTGTATTACAGT	7.8	3	167	CAGGTGAGC	1.8	0.3	0
CTTTATCTTTTTGCAGA	3.4	4	29	CAGGCAAGT	5.4	>4	II
TCTATTGTACTAATAGA	9.3	5	65	CAGGTAAGC	0.9	2.7	I
TTTAAAATTTTCACAGA	8.9	6	104	ACAGTAAGA	4.7	>4	0
CCTTTTTTAAACTCAGG	5.5	7	141	CAGGTGAGT	0.1	>4	0
TTCTTTCTCTCTGCAGA	1.4	8	59	TAGGTAAGT	1.3	4	II
GCATGATGAAATGAAGA	28.2	9	21	TGGGTAATT	5.5	2	II
CCTCTGCTATCTACAGC	4.6	10	248	ATGGTCAGT	4.8	1.3	I
GATTTTTTACCCATAGA	6.1	11	77	GAGGTAAGT	0.9	0.8	0
AAAATTCATTCTTCAGG	9	12	79	CTGGTAAGT	1.5	>4	I
TGTACTTCCTCCACAGA	4.2	13	533	CTGGTGAGT	1.6	2.7	0
ATCTTTTATTTTGCAGC	4.7	14	220	CAGGTAAAA	3.6	>4	I
GCCCCATTTCTTACAGC	5.7	15	199	CAGGTGGGT	2.0	>4	II
TTGCTTTCTTTTGTAGG	3.2	16	73	AAGGTAAAA	3.8	1.6	0
ACTATTTCTCTTTCAGG	3.3	17	78	–	–		

[a] Score of perfect consensus = 0; worst score for acceptors = 42.5, for donors = 30.1.
[b] Phase 0 = position of introns between codons; phase I = interruption after first nucleotide; phase II = interruption after second nucleotide.

Genomic organization of the human IMPG1 gene

As a prerequisite for mutational analyses in human retinopathies, we determined the complete exon/intron organization of the full-length IMPG1 cDNA. Retinal cDNA probes corresponding to nt 164 to 679 and nt 2,340 to 2920 were used to screen a genomic human PAC library, RPCI1. Three independent PAC clones were isolated (dJ47C10, dJ38F21, and dJ69M16). By STS content and restriction enzyme mapping, PAC clones dJ38F21 and dJ69M16 were found to overlap (Fig. 2). By DOP-vector PCR, the T7- and SP6-endclone fragments of PAC dJ38F21 were amplified and used for Southern blot hybridization to *Eco*RI-restricted clones dJ47C10, dJ38F21, and dJ69M16 (data not shown). The 900-bp T7-endfragment of dJ38F21 specifically hybridized to a 7-kb *Hin*cII and a 1.6-kb *Eco*RI fragment of clone dJ69M16, whereas the 800-bp SP6-endfragment of dJ38F21 did not reveal any hybridization signals, indicating that dJ38F21 does not overlap with PAC clone dJ47C10 (Fig. 2).

To identify restriction fragments containing exonic sequences, the full-length IMPG1 cDNA was colony filter hybridized to *Hin*dIII-, *Eco*R I-, and *Sau*3AI-restricted PAC plasmid sublibraries. Positive clones were purified and partially sequenced using the M13 forward and reverse primers, as well as cDNA-derived oligonucleotide primers. Alignment of the genomic and the cDNA sequences revealed the exon/intron boundaries of the human IMPG1 gene. A total of 17 coding exons were identified, ranging in length from 21 to 533 bp and spanning a genomic region of at least 50 kb (Fig. 2 and Table 1). Exon 1 contains the putative translation initiation start codon ATG, conforming to the Kozak consensus sequence (Kozak, 1996), and an additional 151 bp of upstream sequence, corresponding to the most 5'-extending cDNA clone isolated (Kuehn and Hageman, 1995). The start codon is preceded by two in frame termination codons at nt –57 and –60. A 6-kb *Hin*dIII fragment, IPM3'R, isolated from PAC clone dJ38F21, contains exon 17 with the translation stop codon TAA and an additional 745 bp of 3'-untranslated region.

With the exception of exon 4, all acceptor and donor splice sites strictly follow the GT/AG rule (Table 1). The donor splice junction of exon 4 contains a nonconforming "GC" rather than the universal "GT" at this position. Despite this anomaly, the donor splice sequence of exon 4 has a discrimination energy score of 5.4, well within the range expected for true splice junctions (average acceptor score, 5.1; donor score, 3.16) (Table 1).

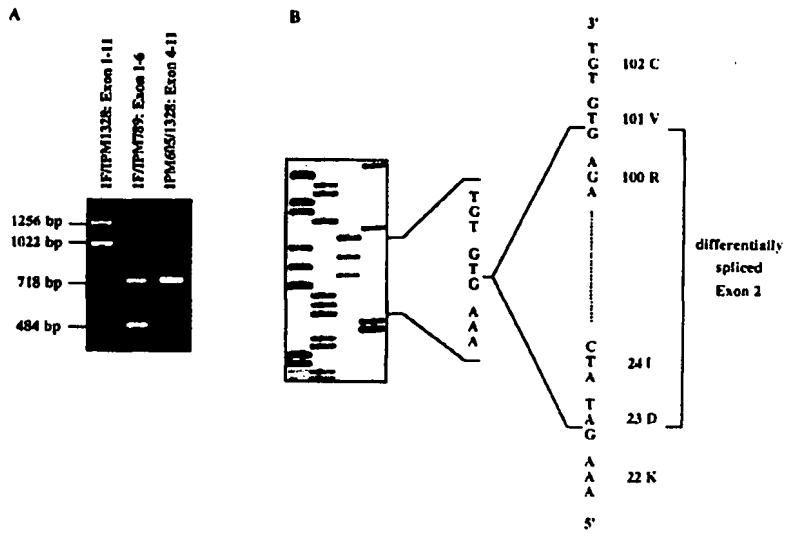

Fig. 3. Alternative splicing of exon 2 in the IMPG1 gene. (**A**) PCR amplification of a human retina cDNA library using primer pairs 1F/IPM1328 and 1F/IPM789 resulted in two distinct bands. The smaller transcript is 234 bp shorter than the expected size. (**B**) Sequencing of the subcloned PCR product 1F/IPM789 revealed splicing of the entire exon 2. As introns 1 and 2 both interrupt a codon after the first nucleotide, skipping of exon 2 does not lead to a frameshift.

(Berg and von Hippel, 1988; Penotti, 1991). The high score of the 3'-splice acceptor sequence of exon 9 has led us to resequence the cDNA and the genomic clone at this position. The cDNA fragment was obtained by PCR amplification from a human λgt10 retinal cDNA library using primers IPM947/IPM1399. This has verified the acceptor splice sequence as shown (Table 1). The approximate sizes of introns 3, 5, 8, 9, 10, 11, 13, and 17 were estimated by PCR amplification using intronic forward and reverse oligonucleotide primers (Table 1). No PCR amplification was observed for the remaining introns, suggesting the presence of introns larger than 4-5 kb.

Alternative splicing of the IMPG1 gene

PCR amplification of the human retinal λgt10 library using primer pairs 1F/IPM1328 (corresponding to exon 1 to 11) and 1F/IPM789 (corresponding to exon 1 to 6) resulted in two different size products (Fig. 3A). Conversely, only a single fragment using primers IPM605/IPM1328 (corresponding to exons 4 to 11) was obtained, suggesting an alternative splice within the first three exons. To test this possibility, the PCR products obtained with 1F/IPM789 were cloned into the pCRII vector, and 40 independent clones were analyzed. Fragments of both 718 bp and 484 bp in size were identified and sequenced (data not shown). Whereas the 718-bp fragment was identical to the known cDNA, the sequencing and comparison of the 484-bp fragment to the known cDNA confirmed an alternative splice event removing the entire exon 2 from the mature RNA (Fig. 3B). Since intron 1 and intron 2 both interrupt the respective codon after the first nucleotide at phase I (Table 1), skipping of exon 2 does not lead to a frameshift (Fig. 3B). Thus, the removal of exon 2 should result in a mature IMPG1 isoform that is missing 78 amino acids.

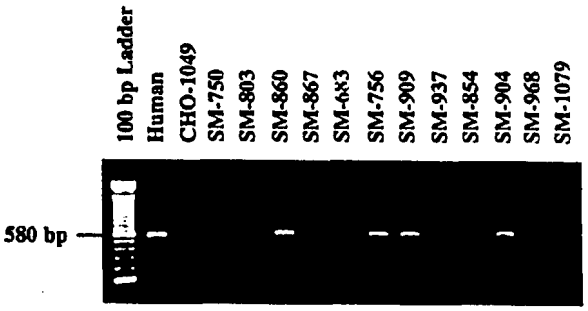

Fig. 4. PCR mapping of the IMPG1 gene using a commercial panel of human × hamster hybrid cell lines. The expected 580-bp PCR fragment was detected in cell hybrids containing human chromosome 6, but not in those lacking this chromosome.

Mapping of IMPG1 to chromosome 6q13→q15

To ascertain the chromosomal location of IMPG1, two independent methods were used. First, genomic DNA from a panel of somatic cell hybrids that retained different sets of human chromosomes was screened using PCR primers C6SP-a/C6SP-m, corresponding to sequences in the 3'-untranslated region of the IMPG1 gene. The PCR product yielded an expected 580-bp fragment in total human genomic DNA and somatic cell hybrids containing human chromosome 6 (SM-756, SM-860, SM-904, and SM-909). In contrast, no PCR product was obtained using DNAs from hybrid cell lines lacking chromosome 6 (GHO-1049, SM-683, SM-750, SM-803, SM-854, SM-867, SM-937, SM-968, and SM-1079) (Fig. 4). The PCR amplification results are consistent with a location of the IMPG1 gene on chromosome 6.

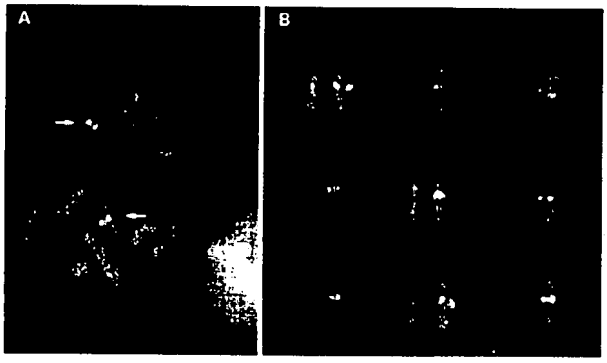

Fig. 5. FISH localization of IMPG1 to chromosome 6q13→q15. (**A**) Partial human metaphase plate after FISH with PAC dJ38F21. Hybridization signals on propidium iodide (PI)-counterstained chromosomes are marked by arrows. (**B**) Examples of hybridization signals observed on selected PI-counterstained human chromosomes 6. The corresponding DAPI-stained chromosomes are shown on the left, respectively.

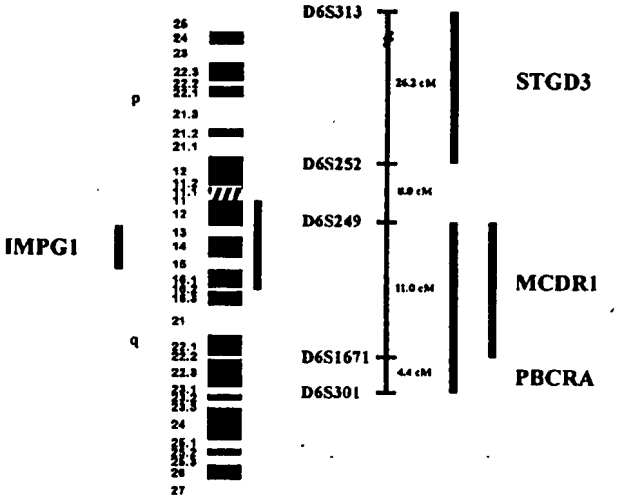

Fig. 6. Diagrammatic representation of the co-localization of IMPG1 and the loci for three maculopathies, STGD3, MCDR1, and PBCRA, on chromosome 6q. FISH hybridization results of IMPG1 are indicated on the left; the genetic locations of the three disorders are shown on the right and have been published previously (STGD3: Stone et al., 1994; MCDR1: Small et al., 1992; PBCRA: Kelsell et al., 1995).

To confirm this assignment and to determine further the subchromosomal location of IMPG1, FISH mapping was performed using a biotin-labeled PAC, dJ38F21. Signals on both chromatids of chromosome 6q13→q15 were repeatedly produced, whereas background signals were distributed randomly (Fig. 5).

Discussion

We report the expression, genomic organization, and chromosome location of a novel interphotoreceptor matrix gene, IMPG1. The selective expression in retinal tissue and the chromosomal mapping of IMPG1 to 6q13-q15 have identified this gene as an attractive candidate for several human macular dystrophies that have previously been localized to 6q11→q16.2 by genetic linkage analysis, viz., autosomal dominant Stargardt-like macular dystrophy (STGD3) (Stone et al., 1994), North Carolina macular dystrophy (MCDR1) (Small et al., 1992), and progressive bifocal chorioretinal atrophy (PBCRA) (Kelsell et al., 1995) (Fig. 6). These three maculopathies are rare hereditary disorders characterized by their mode of inheritance and loss of central vision. In particular, PBCRA is invariantly characterized by subretinal deposits nasal to the optic disc which appear soon after birth and atrophic macular lesions which lead to a progressive reduction in visual acuity and color vision. The expansion of both macular and nasal atrophic lesions toward the optic disc finally leaves only a narrow retinal bridge of relatively intact retina (Godley et al., 1996). In contrast, MCDR1 lesions are highly variable and rarely progress (Small et al., 1993). STGD3 presents with white-yellow macular flecks early in the disease course. Central atrophy develops later and is associated with a progressive loss of central vision in the second or third decade of life (Stone et al., 1994).

The characterization of the exon/intron boundaries of the IMPG1 gene provides the basis for mutational analysis of genomic DNA of affected individuals from the chromosome 6q-linked families. In addition, as STGD3, MCDR1, and PBCRA share some clinical features with other human maculopathies, particularly with age-related macular degeneration, an important cause of visual impairment in elderly patients (Ferris et al., 1984; Young, 1987), it is of importance to test other, as yet unlinked, retinopathies for a possible involvement of IMPG1 in their pathogeneses. The candidate gene approach is most relevant in retinopathies where genetic heterogeneity is a major problem in the identification of the genetic defect (reviewed by Sullivan and Daiger, 1996).

In conclusion, the chromosome mapping and genomic characterization of IMPG1, a novel proteoglycan of the interphotoreceptor matrix, has identified a candidate gene for retinal dystrophies. As the coding sequence of the gene is interrupted by only 16 intervening sequences, the number of exons appears reasonably small in order to enable the mutational analysis of many patients affected with various retinopathies. It is anticipated that the further characterization of function and dysfunction of IMPG1 will shed new light on IPM proteoglycans and their functional involvement in the human eye.

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